REMARKS/ARGUMENTS

Review and reconsideration of the Office Action dated July 28, 2004, is respectfully requested in view of the above amendments and the following remarks.

The pending claims are Claims 1-46.

Claims 1, 4-7, 9-21 and 23-40 were withdrawn in response to the telephone restriction requirement made by the previous Examiner (Examiner Sandals) on June 10, 2003, which was made with traverse. In response to the telephone restriction requirement, Applicant provisionally elected with traverse claims 2, 3, 8, 22 and 41-46 as they read upon SEQ ID NO: 11.

However, the present Examiner, Examiner Maria Marvich, contends that neither the telephone restriction requirement nor the reason for the traversal is of record.

The present Examiner made a subsequent restriction requirement on January 28, 2004 of claims 2, 3, 8, 22 and 41-46 requiring election of one of the sequences of SEQ ID NOs: 5-16 and one of the sequences of SEQ ID NO: 1 or 2. On May 4, 2204, Applicant responded to this later restriction requirement, electing with traverse claims 2, 3, 8, 22 and 41-46 as they read on SEQ ID NO: 11 and SEQ ID NO: 2.

In the instant Office Action the Examiner, upon reconsideration, has removed the restriction requirement as between SEQ ID NO: 1 or SEQ ID NO: 2. However, The Examiner made

final the restriction requirement between SEQ ID NOs: 5-16. The Examiner has also withdrawn claims 42-46 as allegedly reading on a non-elected invention.

In view of the lack of record regarding the telephone restriction requirement and the initial withdrawal with traverse of claims 1, 4-7, 9-21 and 23-40, Applicant withdraws the withdrawal of claims 4-7, 9-21 and 23-40. Furthermore, Applicant has amended claims 4-7, 9-21 and 23-40 so as to directly or indirectly refer back to presently pending claim 2. Care has been taken to ensure that no new matter has been added. Entry of the amendment is requested. Therefore, Applicant respectfully requests the Examiner to reintroduce claims 4-7, 9-21 and 23-40 for examination.

Moreover, Applicant traverses the Examiner's withdrawal of claims 42-46. Applicant respectfully submits that claims 42-46 read upon the combination of SEQ ID NO: 11 and SEQ ID NO: 7. Combination that is within the scope of the election of SEQ ID NO: 11, to the extent that a search of SEQ ID NO: 11 satisfies the requirements for novelty and non-obviousness, no further search of SEQ ID NO: 7 would be required, and the Examiner would not be unduly burdened. SEQ ID NO: 11 and SEQ ID NO: 7 are linked so to make a non-distinct invention.

In addition to claims 2, 3, 8, 22 and 41 which according to the Examiner are under Examination, Applicant respectfully requests the Examiner to further keep under examination the reintroduced claims 4-7, 9-21 and 23-40. Moreover, in view of

the arguments presented above, Applicant requests the Examiner to reconsider his withdrawal of claims 42-46.

Office Action

Turning to the Office Action, the paragraphing of the Examiner is adopted.

Sequence Compliance

The Examiner has required inserting sequence identifiers for the 6 sequences shown in Figure 4. The sequences of Figure 4 are found in the previously submitted sequence listing.

Applicant has amended Figure 4 by moving the "Fig. 4" label to the top of the graphic and inserting the corresponding sequence identifiers according to the sequence listing.

Applicant respectfully submits that the sequences of Figure 4 have been corrected to comply with the requirements of 37 C.F.R. §§ 1.821 through 1.825. Therefore, Applicant respectfully requests the Examiner to withdraw the objection to Figure 4.

Drawings

The Draftsperson has objected to Figure 3 as having incorrect margins and to Figure 1 for having views that are not labeled separately.

Applicant has increased the margins in Figure 3. With respect to Figure 1, Applicant has relabeled the section

corresponding to the restriction map of the plasmid ms23 as "Fig. 1A" and the portion corresponding to the list of enzymes as "Fig. 1B". Furthermore, Applicant has accordingly amended the description of Figure 1 in page 25 of the specification.

Applicant submits herewith replacement sheets with the amended figures 1, 3 and 4. Therefore, Applicant respectfully requests the Examiner to approve the amended drawings and the changes in the specification. If necessary, Applicant will submit formal drawings of Figures 1 and 4 at the time the notice of allowance is received. Accordingly, withdrawal of the rejection to Figures 1 and 3 is respectfully requested.

Specification

The Examiner has objected to the legend of Figure 7 as failing to distinguish parts A and B. The Examiner has also required deleting from the specification, in page 25, lines 4-9, embedded hyperlinks or other form of browser executable code.

Applicant has amended the description of Figure 7 in page 26 of the specification to indicate that Figure 7a and Figure 7b refer to two different experiments and that Figure 7b includes some additional constructs that have been investigated. Applicant has also amended the specification to delete the hyperlinks from the first paragraph in page 25. Applicant has not added new matter.

Applicant respectfully submits that the defects in the specification noted by the Examiner have been corrected.

Therefore, Applicant respectfully requests the withdrawal of the objections to the specification.

Oath/Declaration

The Examiner alleges that under 37 C.F.R. § 1.67(a) the oath or declaration previously submitted is defective. A new oath or declaration identifying the instant application by application number and filing date is required.

Applicant submits a new oath or declaration with the proper application number and filing date under 37 C.F.R. § 1.67(a). Applicant respectfully request the Examiner to approve the oath or declaration submitted herewith.

Claim Objections

The Examiner has objected to claim 41 under 37 C.F.R. § 1.75 as being a substantial duplicate of claim 2. The Examiner's position is explained in the second paragraph of page five of the office action.

The Examiner also objected to claims 2, 3, 8, 22 and 41 as allegedly encompassing non-elected subject matter.

Applicant has deleted claim 41. Applicant notes that objections and rejections to claim 41 are moot in view of its deletion.

Applicant has amended claims 2, 3, 8 and 22 to encompass only matter directed to SEQ ID NO: 11 or SEQ ID NOs: 1 and 2.

Applicant therefore respectfully requests the Examiner to withdraw the objections to claims 2, 3, 8 and 22.

Claims Rejections - 35 USC § 112, second paragraph

The Examiner has rejected claims 2, 3, 8, 22 and 41 under 35 U.S.C.§ 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

With respect to claims 2, 3, 8, 22 and 41, the Examiner contends that the recitation "local gene expression" is unclear. The Examiner position is explained in the second paragraph of page 6 of the office action.

With respect to claim 8, the Examiner alleges that the limitation "at least two of said *cis*-acting elements" lacks sufficient antecedent basis in claim 2.

With respect to claim 22, the Examiner alleges that the metes and bounds of "A *cis*-acting element" are unclear. The Examiner position is found in paragraph 4 of page 6 of the office action.

Applicant respectfully submits that the term "local" is sufficiently defined in the specification at page 3, first full paragraph. Since the specification clearly set forth the meaning of "local gene expression", this term is not unclear as referred in claims 2, 3, 8, 22 and 41. Therefore Applicant

respectfully requests the Examiner to withdraw the rejection to claims 2, 3, 8 and 22 under 35 U.S.C. § 112, second paragraph.

Applicant has amended claim 2 to read "two or more *cis*-acting elements" instead of the recitation "at least one *cis*-acting element". Support for "two or more *cis*-acting elements" can be found in the specification at page 4, lines 2-4 of fifth paragraph. Applicant has not added new matter.

Therefore, claim 2, as amended, has sufficient antecedent basis for the limitation "at least two of said *cis*-acting elements" in claim 8. Consequently, Applicant respectfully requests the Examiner to withdraw the rejection to claim 8 under 35 U.S.C. § 112, second paragraph.

Applicant has amended claim 22, which recites "A *cis*-acting element", to indicate that these terms refer to SEQ ID NO: 11. Therefore, Applicant submits that claim 22, as amended, is not unclear. Applicant respectfully requests the Examiner to withdraw the rejection to claim 11 under 35 U.S.C. § 112, second paragraph.

Claim Rejections - 35 USC 102 § 102

The Examiner rejected claims 2, 22 and 41 under 35 U.S.C. § 102(b) as allegedly anticipated by Van de Locht et al. (Embo J. 1990, vol 9(9):2945-2950). The Examiner contends that Van de Locht et al. teaches a chimeric promoter comprised of the sequence found in SEQ ID NO: 11 and the core of CHS promoter which comprises those elements necessary to initiate

transcription i.e. TATA Box (page 2948, column 1, paragraph 1). The Examiner further notes that the portion of PR2 promoter used in the chimeric construct shown in Figure 6 extends from -168 to -43 which covers the region of SEQ ID NO: 11 as highlighted in Figure 3.

In response, Applicant respectfully submits that Van de Locht et al. identifies a DNA sequence within the PR2-promoter from position -168 to position -52 as being necessary for strong elicitor-inducibility of the promoter (See Van de Locht et al at Abstract and page 2947, left hand column, last paragraph). Furthermore, from the deletion analysis of the promoter (See id. e.g., Figures 5 and 6), Van de Locht et al. teaches that the sequence between positions -168 and -108 is critical for both elicitor-inducibility and the strength of the promoter. On the other hand, the sequence from position -108 to position -52 was considered to mediate elicitor-dependant expression only at a low level (See id. at page 2149, left-hand column, last sentence of the third paragraph and first two sentences of the fourth paragraph).

In the present application, the D-Box sequence (SEQ ID NO: 11) is contained in the promoter sequence shown in Figure 3 of Van de Locht et al. from position -76 to position -46. This means that the D-Box sequence of the present invention is not within a sequence between positions -168 and -108, which is described by Van de Locht et al. as being the most important. Further, the D-Box sequence of the present invention is not even within the sequence range of positions -168 and -52. Thus, Van

de Locht et al. teaches the skilled artisan away from the D-Box sequence (SEQ ID NO: 11) of the present invention.

The D-Box sequence of the present invention (from position -76 to position -46) has been shown later by Rushton et al. (Rushton et al., *The Plant Cell*, 14:749-762 (2002)) that in the form of a tetramer, it mediates a strong pathogen-inducible transcription, whereas the sequence between positions -76 and -52, albeit being pathogen-inducible, only forms a weak promoter. Rushton et al. shows that the sequence between position -76 and -52 is 30 times less strong as a promoter as compared with the sequence between position -76 and -46 (See Rushton et al. at the section bridging pages 755 and 756, as well as, Figure 9; showing that the promoter "4xD" with the additional 6 bases, is 30 times stronger than the promoter 4xD short").

Van de Locht et al. does not teach the *cis*-element D-Box having the sequence shown in SEQ ID NO: 11, as presented in the instant invention. Van de Locht et al. does not teach either a chimeric promoter construct which contains two of the D-Box elements characterized by SEQ ID NO: 11.

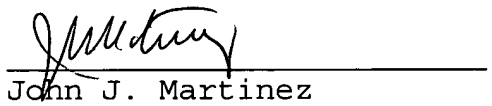
Applicant respectfully submits that in view of above, and the amendment to claim 2 to recite "two or more *cis*-acting elements", Van de Locht et al. does not anticipate claims 2 and 22.

Therefore, Applicant respectfully requests the Examiner to

withdraw the rejection to claims 2 and 22 under 35 U.S.C. § 102(b).

Applicants believe that all the claims are now allowable. Favorable consideration and early issuance of the Notice of Allowance are respectfully requested. Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.

Respectfully submitted,



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Date: **January 27, 2005**

Enclosures: 15 replacement sheets (Drawings), Oath/Declaration, Rushton et al.

ATTORNEY DOCKET NO.: 4038.001

CERTIFICATION OF MAILING AND AUTHORIZATION TO CHARGE

I hereby certify that a copy of the foregoing AMENDMENT A for U.S. Application No.: 09/831,272 filed August 13, 2001, was deposited in first class U.S. mail, postage prepaid, addressed: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on **January 27, 2005**.

The Commissioner is hereby authorized to charge any additional fees, which may be required at any time during the prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account No. 16-0877.

John J. Martinez

IN THE DRAWINGS

Please replace Figures 1, 3 and 4 with the attached amended Figures 1A, 1B, 3 and 4.

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RESEARCH ARTICLE

Synthetic Plant Promoters Containing Defined Regulatory Elements Provide Novel Insights into Pathogen- and Wound-Induced Signaling

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Pathogen-inducible plant promoters contain multiple *cis*-acting elements, only some of which may contribute to pathogen inducibility. Therefore, we made defined synthetic promoters containing tetramers of only a single type of element and present evidence that a range of *cis*-acting elements (boxes W1, W2, GCC, JERE, S, Gst1, and D) can mediate local gene expression in planta after pathogen attack. The expression patterns of the promoters were monitored during interactions with a number of pathogens, including compatible, incompatible, and nonhost interactions. Interestingly, there were major differences in the inducibilities of the various promoters with the pathogens tested as well as differences in the speed of induction and in the basal expression levels. We also show that defense signaling is largely conserved across species boundaries at the *cis*-acting element level. Many of these promoters also direct local wound-induced expression, and this provides evidence for the convergence of resistance gene, nonhost, and wound responses at the level of the promoter elements. We have used these *cis*-acting elements to construct improved synthetic promoters and show the effects of varying the number, order, and spacing of such elements. These promoters are valuable additions to the study of signaling and transcriptional activation during plant–pathogen interactions.

INTRODUCTION

The availability of a range of defined synthetic plant promoters that direct controlled local gene expression in response to pathogens would be a major advance. These promoters could be used to help define signaling pathways, to isolate novel mutants using "targeted genetics" (Hooley, 1998), and to engineer plants with increased disease resistance. The control regions of plant genes are modular and contain a number of *cis*-acting elements, each of which may contribute to one or more aspects of a complex expression profile. One strategy to overcome this complexity is to produce synthetic promoters containing only defined individual elements, thereby reducing expression profile complexity (Salinas et al., 1992). However, although there are numerous reports of synthetic promoters being inducible by elicitors in transient expression systems (Rushton and Somssich, 1998), in most cases it is not known to what extent individual *cis*-acting elements retain their functionality in planta when removed from their native promoter context and whether we can use these individual "modules" to make synthetic promoters that direct a desired expression pattern.

Pathogen-inducible promoters represent an attractive system for the production of synthetic promoters. There are a large number of known pathogen-inducible genes (Rushton and Somssich, 1998), and their promoters are among the best studied in plants. Two groups of pathogen-inducible *cis*-acting elements, the GCC-like elements (Ohme-Tagaki et al., 2000) and the W boxes (Rushton et al., 1996; Eulgem et al., 2000), have been well studied. The GCC box (AGCCGCC) often is found in the promoter regions of defense genes (Ohme-Takagi and Shinshi, 1995). A similar element has been reported to direct jasmonate and elicitor-responsive expression (JERE; AGACCGCC) (Menke et al., 1999), and another (DRE; TACCGAC) directs cold-, salt stress–, and dehydration-responsive expression (Yamaguchi-Shinozaki and Shinozaki, 1994). Recently, another similar GCC-like element called box S (AGCCACC) has been identified that directs expression by fungal elicitors (Kirsch et al., 2000). It appears that minor variations in the core sequences impart responsiveness to different stimuli.

The W box [(T)TGAC(C/T)] is the binding site for members of the WRKY family of transcription factors (Rushton et al., 1996). There is increasing evidence that W boxes are a major class of *cis*-acting elements responsible for the pathogen

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inducibility of many plant genes (Raventós et al., 1995; Rushton et al., 1996; Wang et al., 1998). The importance of W boxes was illustrated recently by studies of the Arabidopsis transcriptome during systemic acquired resistance (Maleck et al., 2000; Petersen et al., 2000). In some cases, clustering of W boxes may be associated with inducibility by pathogens.

Given that GCC-like boxes and W boxes have been so well studied, it is surprising that there is almost no direct in planta evidence that they can mediate pathogen-inducible expression. Although W boxes have been shown to impart elicitor-inducible expression on a minimal promoter in transient expression systems (Raventós et al., 1995; Rushton et al., 1996; Eulgem et al., 1999), there is only one report suggesting that isolated W boxes can function alone in planta and direct pathogen-inducible expression (Kirsch et al., 2001). It was shown that the W box–containing promoter element E17 mediates gene expression at pathogen infection sites in transgenic Arabidopsis plants. Data concerning GCC-like boxes are equally scarce. A synthetic promoter containing four copies of a GCC box directs ethylene-inducible expression in tobacco (Ohme-Takagi and Shinshi, 1995), but pathogen inducibility has yet to be shown.

Both GCC-like elements (Suzuki et al., 1998) and WRKY transcription factors (Hara et al., 2000) have been implicated in gene expression in response to wounding. It was shown recently that wound- and pathogen-induced signaling consists of networks with some shared components (Romeis et al., 1999). It remains an open question, however, whether specific *cis*-acting elements can direct both pathogen- and wound-induced expression in planta or whether these two activities are characteristics of separate elements.

Here, we present a comprehensive study of pathogen-inducible synthetic plant promoters constructed from a range of both well-studied and novel *cis*-acting elements. At least seven different elements can alone direct local pathogen-inducible gene expression in transgenic Arabidopsis plants. Major differences are seen between many of the elements with regard to their background expression, their induction by different pathogens, and their speed of induction. Additionally, we demonstrate that several pathogen-inducible elements also direct local wound-inducible expression and therefore that components of pathogen- and wound-induced signaling are shared.

RESULTS

Elicitor-Inducible Synthetic Promoters

Our approach to making synthetic promoters that are induced locally by pathogens was first to test candidate elements in a parsley transient expression system for inducibility by a pathogen-derived peptide elicitor, pep25. Promising candidates then were introduced into Arabidopsis plants to evaluate their in planta expression patterns and inducibility by pathogens. Figure 1A shows how the synthetic promoters were constructed. Each element was inserted between the SpeI and XbaI restriction sites upstream of the −46 35S minimal promoter of *Cauliflower mosaic virus*.

Initially, synthetic promoters containing tetramers of candidate elements were constructed. The reason for the use of tetramers was the observation that promoters with multimers of elements are stronger than those with just one or

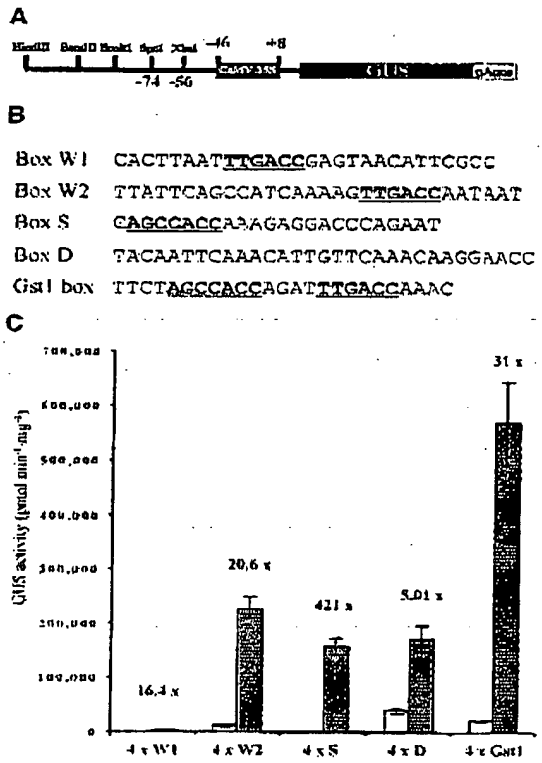

Figure 1. Elicitor-Inducible Synthetic Plant Promoters.

(A) Scheme of the synthetic promoters. Elements were inserted between the SpeI and XbaI sites in pBT10 upstream of the −46 35S promoter of *Cauliflower mosaic virus* (CaMV 35S). pAnos, nos terminator.

(B) Sequence of the elicitor-inducible elements. Core sequences are shown underlined and in boldface.

(C) Elicitor inducibility of the synthetic promoters in a parsley transient expression system. Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM. Qualitatively similar results were obtained upon normalization with a constitutively expressed *Petroselinum crispum UBI4/2* ::luciferase construct (Sprenger-Haussels and Weisshaar, 2000).

two copies (see below). Because it was unknown whether any of the elements were functional in planta, we used tetramers in initial experiments to ensure that any expression would be strong enough to be detectable by β-glucuronidase (GUS) staining. The first elements tested were boxes W1 and W2 from the parsley *PR1* genes (Rushton et al., 1996), box S from the parsley *ELI7* genes (Kirsch et al., 2000), a novel element called box D from the parsley *PR2* gene (P.J. Rushton and K. Hahlbrock, unpublished results), and an element we termed the Gst1 box from the potato *gst1* gene (Strittmatter et al., 1996). The Gst1 box contains an S box and a W box separated by just 4 bp in a region of the promoter that mediates transcriptional activation in response to pathogens, during senescence, and in root apices (Figure 1B). All of the promoters showed inducibility by pep25 in transient expression studies (Figure 1C), although the strength and inducibility of the elements varied greatly. Tetramers of most elements showed inducibilities of five- to 30-fold, whereas four copies of box S had a remarkably high inducibility (>400-fold), which was attributable to an almost complete lack of expression in the absence of pep25.

Although box S is very similar in sequence to the GCC, JERE, and DRE boxes, they appear to direct different patterns of gene expression (Yamaguchi-Shinozaki and Shinozaki, 1994; Ohme-Takagi and Shinshi, 1995; Menke et al., 1999). To investigate these GCC-related elements, we constructed a series of synthetic promoters that are based on box S (Figure 2A). To determine the effect of changes within the core sequence, the flanking sequences remained identical and we altered only the bases necessary to change one variant into another. A construct containing the GCC core sequence was slightly stronger than box S but showed greatly reduced inducibility by pep25 (Figure 2B). This reduction to ~3% of the box S value is remarkable considering that this is the result of a single base pair change and is attributable primarily to an increase in the background level of the GCC box. Results with JERE were almost identical to those with box S, both in strength and fold induction. In contrast, DRE directed a lower level of expression and showed little elicitor inducibility. A nonfunctional version of the GCC box (GCC mut) (Figure 2A) (Ohme-Takagi and Shinshi, 1995) had very low activity but was slightly inducible by pep25. These results show that minor variations in the core sequences of GCC-like elements can have profound effects on both the strength and the elicitor inducibility of these elements.

Expression Patterns of the Synthetic Promoters in Planta and in Response to Wounding

We next addressed the question of whether the synthetic promoters with defined *cis*-acting elements are functional in planta. Promoters containing tetramers of the elements were introduced into Arabidopsis plants. We first determined the levels of background expression for each construct. The majority of the synthetic promoters had little background expression in leaves (Figure 3A and data not shown). By comparison, the background level of expression was sometimes higher in roots (data not shown), and in the case of 4 × W2, this level was very high. Only 4 × D had no appreciable background expression in any parts of the plant.

Many of the leaves shown in Figure 3A manifest local induction by wounding where the leaves have been excised. This response is very rapid, being induced during the time taken to harvest the plants. Therefore, we wounded leaves by cutting through one-half of a leaf and monitored changes in promoter activity. Except for box D, expression from all promoters was induced locally by cutting (Figure 3B and data not shown). Figure 3B also illustrates that this massive damage to the leaf sometimes led to a lower level increase in GUS expression over the entire leaf (cf. Figures 3A and 3B). However, when plants grown in greenhouse conditions exhibited local wound-induced expression, presumably because of insect damage, we never observed expression in

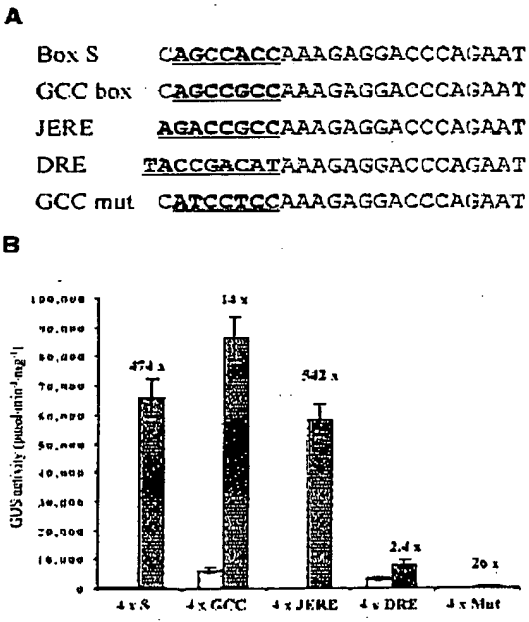

Figure 2. Elicitor-Inducible Promoters Containing GCC-like Elements.

(A) Sequence of the elicitor-inducible GCC-like elements. Core sequences are shown underlined and in boldface.

(B) Elicitor inducibility of synthetic promoters containing GCC-like elements in a parsley transient expression system. Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM. Qualitatively similar results were obtained upon normalization with a constitutively expressed *P. crispum UBI4/2*::luciferase construct (Sprenger-Haussels and Weisshaar, 2000).

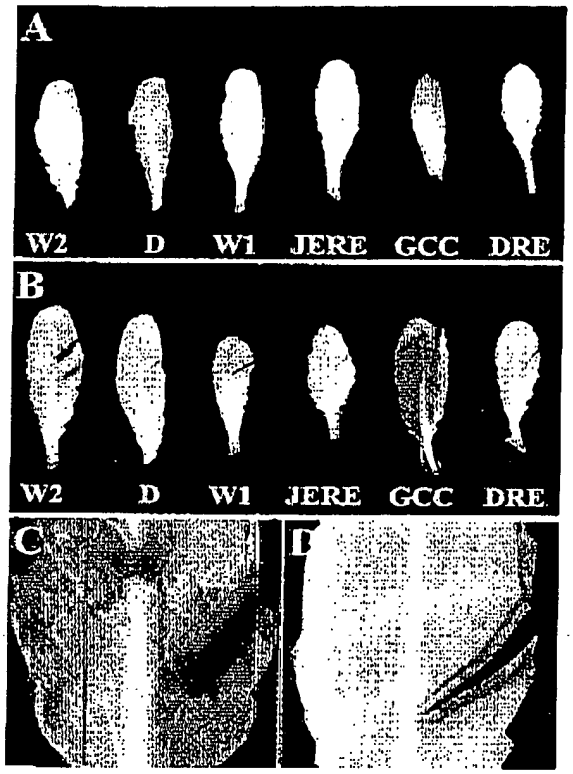

Figure 3. Expression Patterns of Synthetic Promoters in Planta and in Response to Wounding.

(A) Expression patterns (GUS activity) in untreated excised leaves.
(B) Expression patterns 1 hr after wounding by cutting.
(C) Local wound-induced expression from 4 × GCC 1 hr after wounding.
(D) Local wound-induced expression from 4 × S 1 hr after wounding.

other parts of the leaf (data not shown). This finding suggests that local wounding of Arabidopsis plants leads only to highly restricted expression of these promoters. Only when more extensive damage is done (e.g., severing of vascular tissue) is there a response throughout the leaf and subsequent nonlocal induction.

We observed two different patterns of local wound-induced expression after cutting (Figures 3C and 3D). Most common was expression in a layer of cells at the actual cut site (Figure 3C). However, with the same promoters, we sometimes also observed local expression in a ring of cells some distance away from the cut site (Figure 3D). The exact conditions necessary for the production of each pattern were not investigated further.

Synthetic Promoters That Direct Local Gene Expression in Response to *Peronospora parasitica*

Having determined the level of background expression with the synthetic promoters, we looked to answer the following question: Do they direct pathogen-inducible expression? To do so, we investigated their expression during interactions with a range of different pathogens. We inoculated with the biotrophic oomycete *P. parasitica* pv *Cala2*, which produces an incompatible interaction with Columbia (Col-0) plants (Holub et al., 1994). Local gene expression, limited to small areas around infection sites, was seen with 4 × W1, 4 × GCC, 4 × S, 4 × Gst1 (Figure 4), 4 × W2, and JERE (data not shown). By contrast, no expression was detectable with 4 × D. Figure 4 demonstrates that although 4 × GCC is pathogen inducible, it has a much higher level of background expression in uninfected parts than does 4 × S, and these data accurately reflect the transient expression data described above (Figure 2). For all of the tested elements except box D, these results demonstrate that these elements alone can direct pathogen-inducible expression in planta when removed from their native promoter context.

Local Expression during Powdery Mildew Challenge

We next investigated the inducibility of the promoters during the nonhost interaction with the barley powdery mildew *Blumeria graminis* f. sp. *hordei*. Only four synthetic promoters (4 × S [Figure 5A], 2 × W2/2 × S/2 × D [Figure 5B], 4 × GST [data not shown], and 4 × W2/4 × S [data not shown]) directed significant local gene expression under the conditions tested. These promoters all contain box S, suggesting that box S may play a role during nonhost signaling. The germinating spores (sp) form appressorial germ tubes (agt), and although most do not penetrate the plant, local gene expression is found predominantly in the underlying mesophyll cells (Figure 5A) just beneath the attempted penetration site, suggesting that recognition events on the leaf surface trigger defense responses in the underlying cells. Importantly, the level of expression from these promoters and the number of expressing cells correlated directly with the extent of differentiation of the fungus. This is illustrated in Figure 5B, where at one infection site, haustorium initial formation has triggered cell death. Here, a larger number of cells show activation of the promoter, and levels of expression are higher than in the two other infection attempts, in which infection progressed only as far as papilla formation.

During the compatible interaction with the powdery mildew *Erysiphe cichoracearum*, all of the promoters except DRE directed local expression. After 7 to 10 days, areas of powdery mildew growth could be seen, and expression of the synthetic promoters was very high in these infected areas (Figure 5C). When viewed closely, intense GUS staining was apparent in these areas, and a wave of gene expression was apparent in advance of the growing hyphae (Figure 5D).

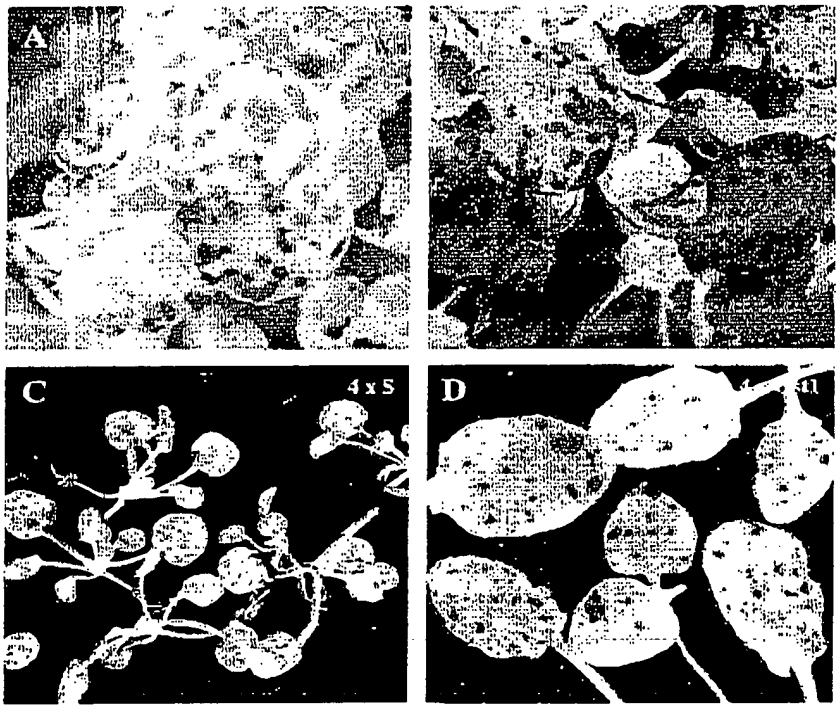

Figure 4. Local Expression in Response to *P. parasitica* pv *Cala2*.

(A) Local GUS expression 2 days after *P. parasitica* infection in Arabidopsis plants containing the synthetic promoter 4 × W1. Blue spots represent infection sites.
(B) Local expression in 4 × GCC plants.
(C) Local expression in 4 × S plants.
(D) Local expression in 4 × Gst1 plants.

Local Gene Expression during Interactions with *Pseudomonas syringae*

We also performed infections with the necrotrophic bacterial pathogen *P. syringae* pv *tomato* using two different isolates: a Col-0–compatible isolate (DC3000) and a Col-0–incompatible isolate (DC3000 carrying the *avrRpm1* gene) (Debener et al., 1991). Figure 6 shows the expression patterns obtained with some of the synthetic promoters. Expression of 4 × W2 was induced locally within 6 hr of infection with the incompatible isolate (Figure 6A). Expression also was apparent with the compatible isolate, although the level of expression was lower. Similar results were obtained with 4 × JERE (Figure 6B), 4 × W1, 4 × S, and 4 × Gst1 (data not shown).

Although untreated leaves from plants containing 4 × GCC showed some background expression, often this was increased greatly by mock infection with $MgCl_2$ (Figure 6D); it was difficult, therefore, to determine whether induction by *P. syringae* was occurring. By contrast, 4 × GCC showed clear induction by *P. parasitica* (Figure 4B), even though background expression was apparent. This finding suggests that the process of injecting liquid into the leaf is itself a sufficient abiotic stress to induce high-level expression of 4 × GCC. Closer inspection of plants containing other synthetic promoters (most notably, 4 × W2, 4 × Gst1, and 4 × W1) showed that background expression levels also are increased sometimes after infiltration of $MgCl_2$ (data not shown). This is similar to the results obtained when a cut was made through one-half of a leaf (Figure 3) and suggests that wounding/stressing a large portion of a leaf can lead to a response in the entire leaf.

Infection of 4 × D–containing plants led to qualitatively different results. No expression was detected in control or mock-infected leaves or with either isolate 6 hr after infection. By contrast, strong local gene expression was seen 24 hr after infection with both isolates (Figure 6C). Again, the level of expression was higher during the incompatible interaction.

Effect of Element Number on Strength and Inducibility

Although the synthetic promoters containing tetramers of elements direct local gene expression upon pathogen attack, many are not yet ideal for our purposes because they have more background expression than desired or they respond to a number of biotic and abiotic stresses. Therefore, we have started to construct improved "second-generation" synthetic promoters using these *cis*-acting elements as building blocks.

The first parameter investigated was the effect of the number of copies of an individual *cis*-acting element in a synthetic promoter. We constructed a series of promoters containing one, two, four, and eight copies of an element and tested these by transient assay. Figure 7A shows the results obtained with 1 × W2, 2 × W2, 4 × W2, and 8 × W2. Increasing the number of copies of W2 increased the strength of the promoter progressively. However, 2 × W2 had the best inducibility, because additional copies caused a proportionally greater increase in background expression. Similar results were obtained with boxes S and D (data not shown).

These findings suggest that promoters containing fewer copies of an element may be better suited to mediate pathogen-specific inducibility in planta. Accordingly, we introduced promoters with two copies of elements into Arabidopsis plants. Figures 7B and 7C show a comparison of 2 × S and 4 × S plants treated with *P. syringae* and demonstrate that the differences between the two promoters that were observed in the transient assay also are apparent in planta. Both promoters were inducible by pathogens, but 4 × S showed a higher background level and clear wound induction where the leaf was excised. By contrast, 2 × S, al-

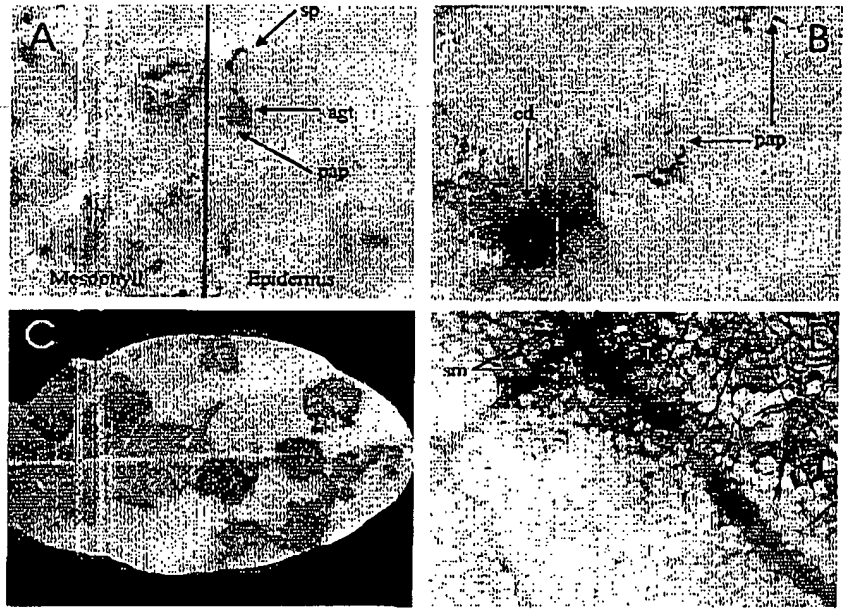

Figure 5. Local Expression during Nonhost and Compatible Powdery Mildew Interactions.

(A) Light micrograph of one leaf infection site on a 4 × S plant viewed at two different planes of focus. The epidermal plane (right) shows the inducing penetration attempt by a germinated *B. graminis* spore (sp). The differentiation of an appressorial germ tube (agt) coincides with local cell wall thickening (pap). Focusing deeper into the tissue (mesophyll plane; left) reveals GUS expression in mesophyll cells just underneath the penetration attempt. The photograph was taken 2 days after inoculation with the nonhost pathogen *B. graminis*. Staining of the fungus was with Coomassie blue.

(B) Local expression in a 2 × W2/2 × S/2 × D plant upon *B. graminis* challenge. The rare successful penetration event at left triggered a cell death response (cd), whereas early aborted penetration attempts correlated with papilla formation (pap).

(C) Local expression 7 days after infection with the compatible powdery mildew *E. cichoracearum* in a 4 × S plant. Blue spots represent infection sites.

(D) Closeup of the border region of an infection site from (C). Reporter gene expression coincides with superficial mycelium (sm).

though clearly pathogen inducible, showed on average less background and in many cases no apparent wound induction.

Spacing Effects and Promoter Strength

Our data show that not all W box–containing synthetic promoters behave similarly. For example, both transient expression experiments (Figure 1) and results from transgenic plants show that box W2 is much stronger than box W1, even though both contain the same TTGACC core element. Therefore, we asked the question: What makes a W box strong or weak? To eliminate possible spacing effects, we made new versions of both box W1 (box W1 new) and box W2 (box W2 new) that are identical in length and have TTGACC core sequences in identical positions (Figure 8A). The only difference between the new version of box W1 and the original is the addition of the preceding 7 bp from the *PR1-1* promoter. To our surprise, both of the new elements were strong, with no detectable difference between 4 × W2, 4 × W2 new, and 4 × W1 new (Figure 8B). The seven additional bases in box W1 new have increased the strength either by adding a positive element or by altering the spacing between TTGACC core elements. Therefore, we made a version of 4 × W1 new in which an unrelated sequence was substituted for the original seven bases (Figure 8A). This construct (4 × W1 5 prime mut) directed a similar level of expression to 4 × W1 new (Figure 8B). Thus, the difference in strength between the two versions of box W1 was not sequence dependent, excluding the possibility that a new *cis*-acting element was generated. The pronounced difference in strength between 4 × W1 and 4 × W2, therefore, seems to be a spacing effect caused by different distances between core TTGACC elements.

To establish whether spacing from the TATA box had any effect on promoter strength or inducibility, we inserted elements 18 bp farther upstream into the SpeI site instead of between the SpeI and XbaI sites (Figure 1A). Figure 8C shows that this resulted in a small increase in strength but had little effect on inducibility. Placing the elements at other positions farther upstream had little effect (data not shown). Together, our results suggest that positioning an element closer to the TATA box can result in some change in promoter strength but that there is little effect on inducibility.

Box D and the Role of Flanking Sequences

Box D is of considerable interest because box D–containing synthetic promoters have no appreciable background expression or wound inducibility in planta, and they also show different kinetics of induction by pathogens. Box D was discovered as a DNaseI footprint from approximately −76 to −52 in the parsley *PR2* promoter (P.J. Rushton and K. Hahlbrock, unpublished results). This region (box D short)

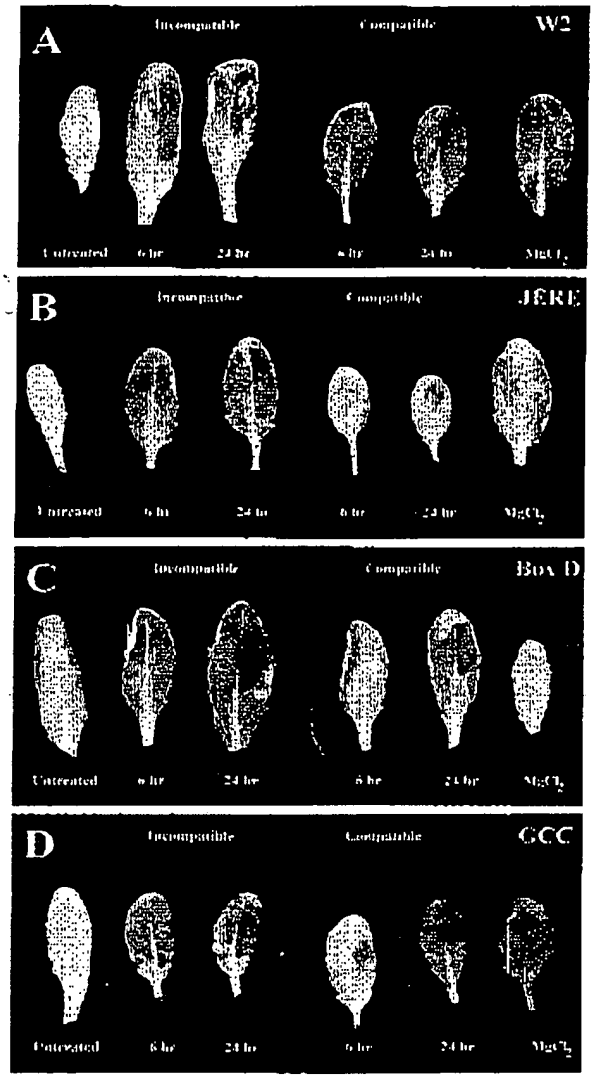

Figure 6. Expression during Interactions with *P. syringae*.

Using a syringe, leaves were inoculated with either isolate DC3000 (compatible interaction) or isolate DC3000 carrying the *avrRpm1* gene (incompatible interaction) and were harvested after 6 or 24 hr. As controls, untreated leaves and leaves 24 hr after treatment with $MgCl_2$ buffer were harvested and stained for GUS activity.
(A) 4 × W2.
(B) 4 × JERE.
(C) 4 × D.
(D) 4 × GCC.

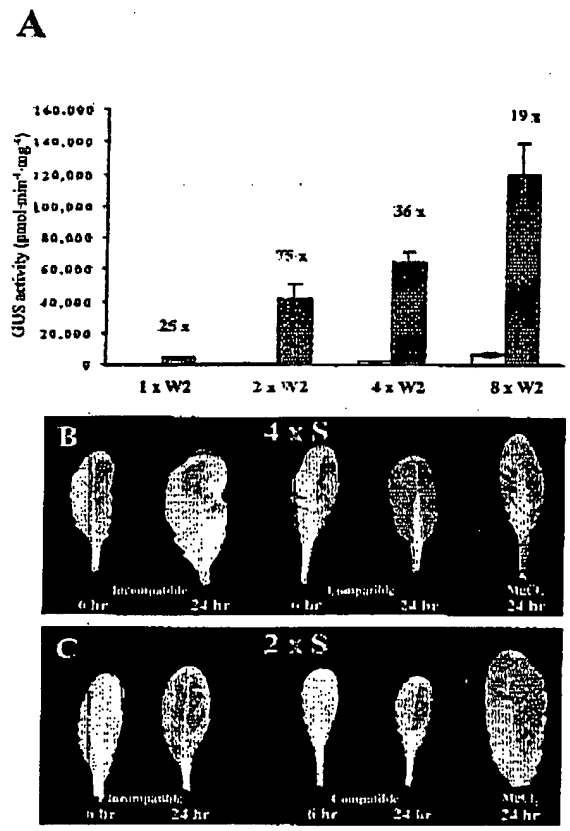

Figure 7. Effect of Element Number on Strength and Inducibility.

(A) Elicitor inducibility of synthetic promoters containing increasing numbers of the elicitor-inducible *cis*-acting element box W2. Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM.

(B) and (C) Expression patterns with plants containing the synthetic promoters 4 × S (B) and 2 × S (C) during interactions with *P. syringae*. Leaves were inoculated with either isolate DC3000 (compatible interaction) or isolate DC3000 carrying the *avrRpm1* gene (incompatible interaction) and were harvested after 6 or 24 hr.

(Figure 9A) had a high elicitor inducibility but was weak (Figure 9B). Because the exact extent of the element was unclear, a longer version (box D) containing the next six bases from the *PR2* promoter at the 3′ end was constructed (Figure 9A). Box D was almost 30 times stronger than 4 × D short (Figure 9B), although inducibility was reduced as a result of increased background levels. We then constructed a version of box D in which the six additional bases were exchanged (Figure 9B, box D mut), and this was almost identical to box D short (Figure 9A), demonstrating that the considerable increase in strength is a sequence-specific effect that is dependent on the six bases GGAACC. Therefore, we believe that box D consists of at least two elements: an elicitor-responsive element and a positive-acting element. This positive element cannot function alone (Figure 9, 4 × D 3 prime and 4 × D 3 prime mut) but appears to be a coupling element that forms a functional unit with the elicitor-inducible element(s). Alternatively, the shortened version of box D simply may lack crucial 3′ nucleotides of a single element required for selective high-affinity binding of a specific transcription factor, thus allowing binding to other related factors, leading to the observed differences in inducibility.

Promoters with Combinations of Elements

Having taken a reductionist approach, reducing synthetic promoters to a single type of *cis*-acting element to demonstrate functionality, we next took the first steps toward making improved synthetic promoters that contain more than one type of defined *cis*-acting element. The question that we asked was the following: What effect does the insertion of a second element have? To answer this question, we inserted four copies of box S either upstream (4 × S/4 × W2) or downstream (4 × W2/4 × S) of 4 × W2. Figure 10A shows that this insertion had little effect on the strength of the promoters and a relatively minor effect on inducibility. Mixing up the elements to make 2 × S/2 × W2/2 × S/2 × W2 led to only a slight increase in promoter strength.

In contrast to the transient expression results, in planta studies produced encouraging results. We introduced two synthetic promoters (2 × W2/2 × S/2 × D and 4 × W2/4 × S) that contain combinations of different elements into Arabidopsis plants. Both showed good inducibility by a range of pathogens and were among the best promoters tested, combining high inducibility with low background (Figures 10B and 10C). It seems likely that all of the *cis*-acting elements contribute to the overall expression of the synthetic promoter and that promoters containing carefully selected combinations of elements may be among the best pathogen-inducible promoters.

DISCUSSION

Pathogen-Inducible Synthetic Promoters

In this report, we provide direct evidence that a range of pathogen-inducible *cis*-acting elements can alone mediate pathogen-inducible expression in planta. When taken out of their native promoter contexts, they retain pathogen inducibility as components of synthetic promoters, directing expression that is local and that correlates with the extent of

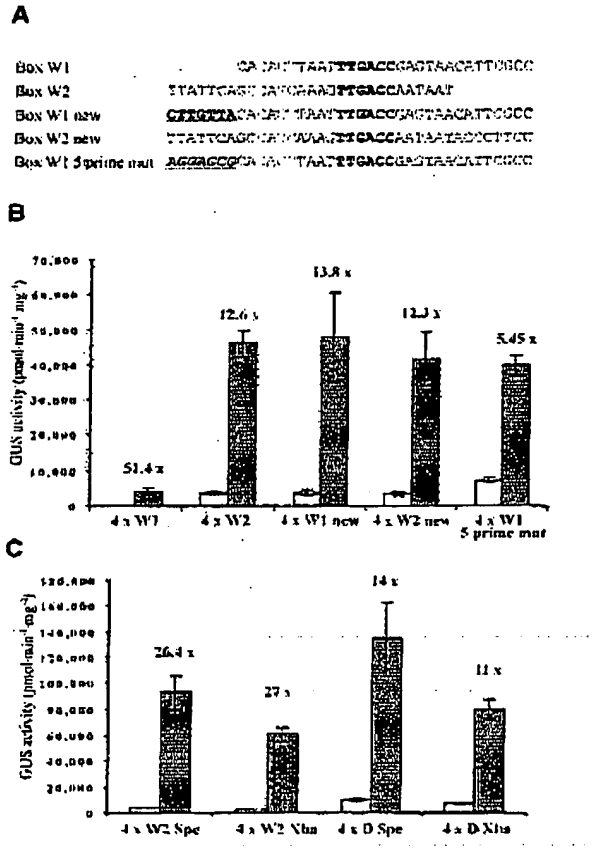

Figure 8. Spacing Effects and Promoter Strength.

(A) Sequences of the different versions of box W1 and box W2. W box core sequences are indicated in boldface. The seven additional bases in box W1 new are shown in boldface and underlined. The seven unrelated bases in box W1 5 prime mut are shown in boldface italics and underlined.

(B) Elicitor inducibility of the synthetic promoters shown in (A). Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM.

(C) Elicitor inducibility of synthetic promoters with elements inserted at different positions. Elements were inserted into either the SpeI site (−74) or the XbaI site (−56).

growth of the pathogen. The *cis*-acting elements tested fall into three groups: W boxes, GCC-like boxes, and box D. Our observations suggest that binding sites for WRKY (W box) or AP2/ERF (GCC-like box) transcription factors can be sufficient to confer pathogen inducibility on a promoter. This is an important observation because these represent two of the three largest families of plant-specific transcription factors (Riechmann and Ratcliffe, 2000). It will be interesting to extend these studies to include other pathogen-inducible elements such as Myb Recognition Elements (MREs) and *as-1*–like elements, although evidence suggests that these elements might not be able to function alone (Rushton and Somssich, 1998).

Interestingly, although more than one type of *cis*-acting element is not required for pathogen inducibility, some pathogen-inducible promoters contain elements of more than one type. An example is the Gst1 box, which contains both a W box and an S box. This places the *gst1* gene under the control of both WRKY and AP2/ERF transcription factors. The potato *gst1* promoter has been well studied (Strittmatter et al., 1996), and our work provides the first clear evidence of how this gene is activated transcriptionally in response to pathogens. It may be common that signaling pathways operating via different transcription factors can target the same gene; another example is the parsley *WRKY1* gene (a W box and a GCC box) (Eulgem et al., 1999).

Our results suggest that defense signaling is largely conserved across species boundaries, because an element from a potato gene (the Gst1 box) is active in a parsley transient expression system and in Arabidopsis plants. In fact, none of the elements tested originate from Arabidopsis promoters, yet all retain their functionality. These results also validate our use of a parsley transient expression system for the initial characterization of elements, because we have successfully identified and characterized numerous pathogen-inducible elements and synthetic promoters in a way that would not have been possible in planta. In almost all cases, elicitor inducibility in the transient expression system is accompanied by pathogen inducibility in planta.

Signaling in Different Types of Plant–Pathogen Interactions

Table 1 summarizes the responses of the synthetic promoters to pathogens under the conditions tested. Importantly, not all of the elements respond in the same way to pathogens and wounding. Even elements that are bound by the same family of transcription factors (e.g., GCC, JERE, S, and DRE) show different expression patterns. This is consistent with reports that these elements may respond to different hormones, for example, salicylic acid (W boxes) (Yang et al., 1999), jasmonate (JERE) (Menke et al., 1999), and ethylene (GCC) (Ohme-Takagi and Shinshi, 1995). Therefore, we have generated a spectrum of synthetic promoters useful for studying plant–pathogen interactions at the molecular level.

During compatible interactions, the synthetic promoters accurately report the in vivo situation in which gene activation is slower and perhaps weaker (Lo et al., 1999). In this context, the observed high-level expression of the synthetic promoters during the compatible interaction with a powdery mildew (*E. cichoracearum*) illustrates their potential

A

Box D short	TACAATTCAAACATTGTTCAAACAA
Box D	TACAATTCAAACATTGTTCAAACAAGGAACC
Box D mut	TACAATTCAAACATTGTTCAAACAACTTGTT
D 3 prime	CAAGGAACCT
4 x D 3 prime mut	CAACTTGTAT

B

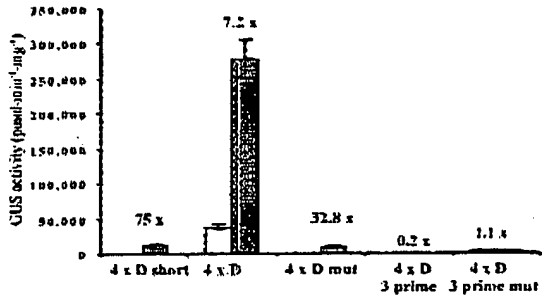

Figure 9. Box D.

(A) Sequences of the different versions of box D. The six additional bases in box D are shown in boldface and underlined. The six unrelated bases in box D mut and 4 x D 3 prime mut are shown in boldface italics and underlined.

(B) Elicitor inducibility of the synthetic promoters shown in (A). Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM.

usefulness during plant–pathogen interactions of commercial importance.

Under the conditions tested, only promoters containing box S consistently showed local expression during the nonhost interaction with barley powdery mildew. Box S, therefore, could be a useful tool for studying nonhost responses and for engineering plants with broad-spectrum resistance. These results are remarkable considering that there is only a single basepair difference between box S and the GCC box (Figure 2). This single alteration results in box S having much lower background levels both in the transient expression system and in planta. Given that both elements probably are bound by AP2/ERF transcription factors, experiments designed to identify family members with high affinities for box S could yield useful data concerning signaling during plant defense.

Box D is an extremely interesting novel element, unlike any of the others tested, because it combines an apparent lack of background expression or induction by wounding with strong induction during some, but not all, plant–pathogen interactions (Table 1). Box D has been found to be responsive to *P. syringae*, *E. cichoracearum*, the bacterially derived peptide elicitor flg22 (data not shown), and the oomycete-derived peptide elicitor pep25 in protoplasts. Box D also responds with different kinetics than other elements, being induced later than the other elements (Figure 6). The molecular characterization of box D and the identification of its cognate transcription factors would provide new insights into defense gene activation during plant–pathogen interactions.

Pathogen and Wound Signaling through the Same *cis*-Acting Elements

Recently, work on plant defense signaling has demonstrated the convergence of resistance gene, elicitor, wound, and salicylate responses at the level of mitogen-activated protein kinase (MAPK) activation (Romeis et al., 1999). It is not clear at present whether these signaling pathways merge at the MAPKs or upstream thereof or whether the same MAPK can mediate disparate responses by interacting with other proteins (Bent, 2001). Our results showing that tetramers of a range of pathogen-inducible *cis*-acting elements also direct local wound-induced gene expression demonstrate the convergence of resistance gene, nonhost, and wound responses at the level of promoter elements. This observation is in agreement with recent data demonstrating an extensive overlap in the transcriptional response of plants to race-specific elicitors and mechanical stress (Durrant et al., 2000). Studies of 290 *Avr9/Cf-9* rapidly elicited genes demonstrated that many of these also are induced by wounding (e.g., by cutting or infusion of liquids).

The results presented here extend these observations from the level of the entire promoter to that of individual *cis*-acting elements. In addition, the identities of the elements suggest that pathways involving salicylic acid, jasmonate, and ethylene may converge at this level. One key question that remains is whether the same or different members of transcription factor families are responsible for both pathogen and wound responses. In other words, whether convergence occurs at the level of transcription factors as well as at the level of *cis*-acting elements and promoter architecture. Most likely, these signaling networks have shared components, with proteins at many nodes being capable of receiving inputs from multiple pathways (Bent, 2001). Many pathogen- and wound-inducible *cis*-acting elements could represent such nodes.

Improving Synthetic Promoters

We used synthetic promoters containing tetramers of elements to ensure that the promoters were strong enough to detect activity by GUS staining. These promoters, however, are not optimal for all purposes; therefore, we set about making improved second-generation promoters by varying

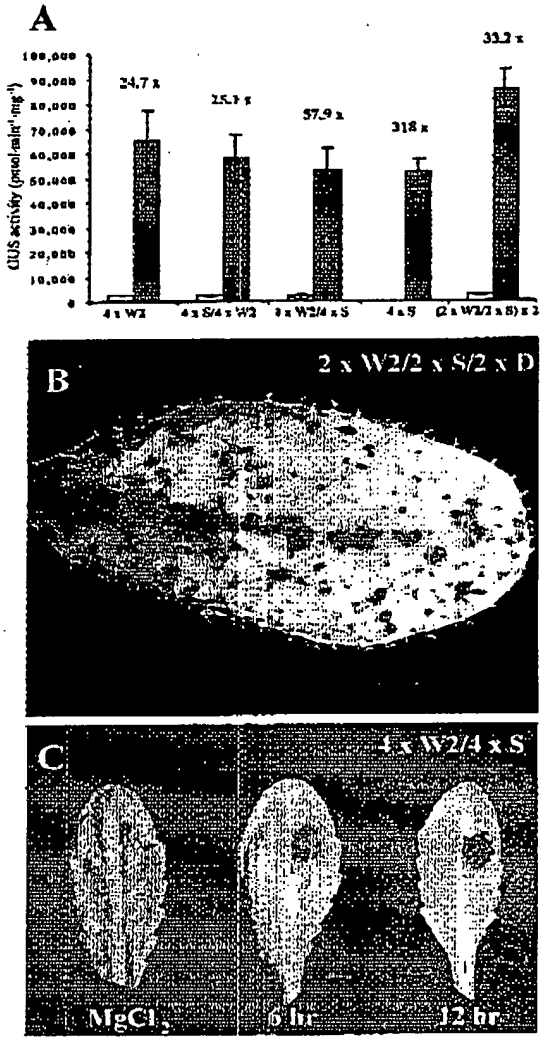

Figure 10. Synthetic Promoters Containing Combinations of Elements.

(A) Elicitor inducibility of synthetic promoters containing combinations of box W2 and box S. Gray bars represent GUS activity 8 hr after pep25 addition. White bars show the level of GUS activity in the absence of pep25. The fold inducibility is shown, and error bars indicate ±SEM.

(B) GUS expression pattern of the synthetic promoter 2 × W2/2 × S/2 × D 3 days after treatment with *B. graminis* (nonhost interaction).

(C) GUS expression pattern of the synthetic promoter 4 × W2/4 × S 6 and 12 hr after treatment with *P. syringae* carrying the *avrRpm1* gene (incompatible interaction).

several parameters. Most important was the number of copies of an individual element in a promoter. Both the strength and the inducibility of a promoter can be modulated by varying the number of copies of an element. Importantly, this also can have the effect of reducing/eliminating some background expression because pathogen inducibility appears stronger than basal or wound-induced expression (Figure 7B). Spacing between individual *cis*-acting elements and/or between these elements and the preinitiation complex also can have a profound effect (Figure 8), but spacing is difficult to predict (Wray, 1998), and the optimal spacing, like the optimal number of elements, needs to be determined experimentally.

Promoters with combinations of different elements may be among the best pathogen-inducible promoters (Figures 10B and 10C), because they often combine good inducibility with low background. Together, our results suggest that the optimal pathogen-inducible synthetic promoters may consist of combinations of one or two copies of defined *cis*-acting elements. Perhaps the best synthetic promoters, although made up of carefully chosen components, will not be that dissimilar to natural promoters after all.

Biological Importance and Applications

The pathogen-inducible synthetic promoters could have major applications, first, as molecular markers, and second, in engineering crops with increased disease resistance. As molecular markers, the promoters are attractive because they consist of one *cis*-acting element and therefore one defined end point of signaling pathways. This could have major advantages over the full-length promoters that are used commonly. Through introduction into mutant backgrounds, the synthetic promoters can be used to better characterize these mutants, and the use of defined synthetic promoters for this purpose could become a standard practice. Differences in responses during different plant–pathogen interactions also can be investigated at the molecular level. Synthetic promoters can be used as reporters for mutant screens using targeted genetics (Hooley, 1998), and this may prove a powerful approach.

The spread of plant pathogens and insect pests is increasing worldwide (Moffat, 2001). Researchers have identified numerous plant and pathogen genes that can be used to increase crop resistance toward invading pathogens. These strategies involve interfering with the replication of viruses in the plant, expression of gene products toxic to certain pathogens, and enhancement of the plant's own natural resistance mechanisms. Such introduced genes usually are placed under the control of strong promoters, yielding constitutive expression of the gene product in all tissues of the plant. This can have detrimental effects on plant growth, development, and crop yield. Use of the synthetic promoters presented here and future improvements thereof may prove valuable in engineering plants with increased resistance,

Table 1. Expression Patterns of Synthetic Promoters

Synthetic Promoter	*P. parasitica* Incompatible	*P. syringae* Incompatible	*P. syringae* Compatible	*E. cichoracearum* Compatible	*B. graminis* Nonhost	Wounding Abiotic
4 × W2	+	+	+	+	−	+
4 × W1	+	+	+	+	−	+
4 × D	−	+	+	+	−	−
4 × GCC	+	±	±	+	−	+
4 × S	+	+	+	+	+	+
4 × JERE	+	+	+	+	−	+
4 × GST	+	+	+	+	+	+
4 × DRE	ND	−	−	−	−	+
4 × W2/4 × S	+	+	+	+	+	+
4 × W2/2 × S/2 × D	+	+	+	+	+	+

(+), high-level induction; (±), lower level of induction attributable to lower induced expression or high background; (−), no expression; ND, not determined.

because the use of such defined regulatory sequences may allow highly restricted expression of the desired gene product exclusively at the sites of attempted pathogen invasion. Thus, expression of the gene product is limited to cells surrounding an infection site and is not found in healthy parts of the plant. Moreover, expression via such promoters can be triggered by a range of different pathogens, including during compatible interactions with pathogens of commercial importance, such as powdery mildew. This expression may be sufficient to abort the progression of the invader even in such compatible interactions.

METHODS

Construction of Synthetic Promoters

Promoter constructs were produced by annealing phosphorylated upper and lower strand oligonucleotides to create elements containing a SpeI restriction site at the 5′ end and an XbaI restriction site at the 3′ end. These were introduced into pBT10–β-glucuronidase (GUS) (Sprenger-Haussels and Weisshaar, 2000) between the SpeI and XbaI sites (Figure 1). Promoters containing multiple copies of elements or combinations of elements in any desired order were obtained by digesting the constructs with either SpeI or XbaI together with SacI, which cuts the plasmid at a site outside of the synthetic promoter. Ligation of two such fragments recreates the plasmid with an increased number of elements. This can be repeated as the 5′ SpeI and the 3′ XbaI sites are recreated, but internal SpeI-XbaI ligations result in the loss of these restriction sites. For analysis in *Arabidopsis thaliana*, the entire synthetic promoter was excised as a HindIII-SacI fragment and ligated into the binary vector pGPTV-GUS-KAN (Becker et al., 1992).

Transient Expression

Transient expression analysis was performed as described previously (van de Löcht et al., 1990) using 20 μg of ScaI-linearized DNA per assay. Protoplasts were harvested 8 hr after transfection. All results represent a minimum of seven independent experiments. Normalization control experiments were performed after cotransfection with a constitutively expressed *Petroselinum crispum UBI4/2*::luciferase construct (Sprenger-Haussels and Weisshaar, 2000).

Transgenic Arabidopsis Lines

Agrobacterium tumefaciens–mediated plant transformation was performed as described (Bechtold et al., 1993). Approximately 10 independent lines were isolated for each synthetic promoter-GUS reporter transgene, and between two and four representative lines (typically between T2 and T5) were subjected to detailed analysis. Tested lines varied between T2 and T5, depending on the promoter tested. Histochemical staining for GUS activity was performed as described (Jefferson, 1987).

Wounding of Plants

Leaves were cut with scissors and harvested 1 hr thereafter. Control leaves were excised and immediately stained for GUS activity.

Infection of Plants with Pathogens

Plants were infected with *Pseudomonas syringae* pv *tomato* strain DC3000 without an *avr* gene (compatible) or with the *avrRpm1* gene (incompatible) or with *Peronospora parasitica* pv *Cala2* as described (Kirsch et al., 2001) and stained for GUS activity. Four- to 6-week-old Arabidopsis plants were infected with *Erysiphe cichoracearum* UCSC1 according to the method of Vogel and Somerville (2000) and with *Blumeria graminis* f. sp. *hordei* K1 according to Peterhänsel et al. (1997).

Microscopic Analyses

Clearing of leaves and staining of fungal structures were performed according to Peterhänsel et al. (1997).

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